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ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC BACTERIA ISOLATED FROM MANGROVE PLANT

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ABSTRACT

"A chemical substance derived from microorganisms, which has the capacity of inhibiting growth and even destroying other organisms in dilute solutions is called as antibiotic" was introduced by Selman and Waksman in 1942. Endophytes - Microbes that colonize living internal tissues of plants without causing any immediate, overt negative effect. In this present study we collected roots, stems and leaf samples of mangrove plant in sterile cover from pichavaram mangrove forest, Chidambaram, Tamilnadu, India. Samples transferred to lab and processed immediately after surface sterilization by standard procedure. Isolation and purification of endophytic bacteria was done by using starch casein agar with antibiotics to inhibit the growth of fungi. *In vitro* screening was done to identify antibacterial activity of endophytic bacteria by agar well diffusion method with 9 different clinical pathogens *viz., Staphylococcus aureus, Escheriachia coli, Proteus vulgaris, Proteus mirabilis, Klebsiella species, Pseudomonas fluorescese, Pseudomonas auruginosa, Salmonella typhi* and *Streptococcus pyogens.* Totally 24 isolates were recovered from all the samples. 9 isolate from root, 8 isolate from stem and 7 isolate from leaf samples. Up on that 4 isolate from root, 2 isolate from stem and 2 isolate from leaf shows best activity against most of the clinical pathogens.

KEY WORDS

Endophytes, Mangrove, Pichavaram, Clinical pathogens and Antibacterial activity.

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INTRODUCTION

The phrase bioprospecting is today most frequently used to describe the collection and screening of biological material for commercial purposes[1]. Natural product based compounds have an immerse impact on modern medicine since about 40% of prescription drugs are based on them[2].

Since, the discovery of endophytes by Darnal, Germany in 1904[3]. Various investigations had done on

endophytes in different ways, which is usually dependent on the prospective from which the endophytes were being isolated and subsequently examined.

[4]Bacon and white (2000) give an inclusive and widely accepted definition of Endophytes - "Microbes that colonize living, internal tissues of plants without carrying any immediate over negative effects".

Endophytes enter plant tissue primarily through the root zone; however, aerial portions of plants such as flowers, stems and cotyledons, may also be used for entry[5]. Specifically the bacteria enter tissues via germinating radicals secondary roots, stomates or as a result of foliar damage[6]. Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant[7].

In general endophytic bacteria occur at lower population densities that rhizospheric bacteria or bacterial pathogens[7,8].

A review of published endophytic bacteria was reported by Hallmann and his associates in 1997, but the list is no longer complete, as there is much interest in this area and new endophytes are continuously being reported.

Endophyte is noteworthy that of the early 3, 00,000 plant species that exist on the earth, each individual host to one or more endophytes[2].

Secondary metabolites produced by endophytes provide a variety at fitness enhancements such as increased resistant to herbivora, parasitism trough as well as growth enhancements[9].

Recently many known as well as new endophytic bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, anti-inflammatory, antioxidant etc., have been identified[2].

A specific rationale for the collection of each plant for endophyte isolation and natural product discovery is used[2]. Several reasonable hypothesis govern this plant selection strategy and these are as follows.

1. Plants from unique environmental especially those with an unusual biology.
2. Plants that have an ethnobotanical history.
3. Plant that are endemic that has an unusual longevity.
4. Plant growing in areas of great bio diversity also here the prospect of housing endophytes with great biodiversity.
5. Mangroves are salt tolerant plants existing at the interface between land and sea in the tropical and subtropical latitudes[10].

However, the mangrove ecosystem is a largely unexplored source for actinomycetes with the potential to produce biologically active secondary metabolites.

The above plant have different ethnobiological history, in this present study we select a mangrove tree with great biodiversity for this research.

MATERIAL AND METHODS

Sample collection and transport

Samples were collected from pichavaram and Annamalai nagar. All the Samples were collected in a sterile plastic covers, transferred to laboratory and processed immediately.

Sample pretreatment and endophytic bacterial isolation

For the pretreatment of samples and isolation of endophytic bacteria, the method described by (Sun *et al*, 2006)[11] was adopted with some modifications. All the samples were excised and subjected to a three step surface sterilization procedure. All the samples were cut into bits (0.5-1.0 cm), Washed in running tap water and Rinsed in 70% ethanol for 30 sec then Rinsed in sodium hypochloride (3-5%) for 3 min finally Washed in sterile water 3 times thoroughly.

Plated on starch casein agar and nutrient agar with nystatin and cycloheximide (50µg/ml) to suppress fungal growth and Incubated at 28ºC for 3 days after incubation plates were observed for the growth of endophytic bacteria. Morphologically different colonies were selected, Pure culture were prepared and stored in refrigerator.

Antimicrobial activity

For the preparation of 18 hrs culture, nutrient broth was prepared and all the bacterial isolates were inoculated and incubated at 28°C for the production of antimicrobial compounds by using no 3 medium[12] was prepared and about 10% of inoculums was

transferred into it. All the test tubes were incubated in rotary shaker with 95 rpm for 120 hours at 28°C. After incubation, 2 ml of culture broth was taken and separated by centrifugation at 10,000 rpm for 10 minutes. After centrifugation the endophytic culture supernatant was collected and used for antimicrobial activity testing.

Antimicrobial activity of endophytic culture supernatant was tested by agar well diffusion method using nutrient agar medium. Test bacterial strains used in this study include human pathogens *Staphylococcus aureus, Escheriachia coli, Proteus vulgaris, Proteus mirablis, Klebsiella species, Pseudomonas fluorescese, Pseudomonas auruginosa, Salmonella typhi* and *Streptococcus pyogens.* All the cutures were obtained from Department of Microbiology, Rajah sir muthiah medical college and hospital, Chidambaram, Tamilnadu, India.

18 hours broth cultures of test organism were inoculated into Muller Hinton agar using sterile cotton swab. About 5 mm size well was made using sterile cork borer and 100 µl of culture supernatant was added into it. All the plates were inoculated at 37°C for 24 hours.

Observed for the zone of inhibition or clearance after incubation.

Medium (antimicrobial substance production)

Poly peptone – 10.0g
Glucose – 10.0g
Potassium dihydrogen phosphate – 1.0g
Magnesium sulphate – 0.5g
Distilled water – 1000ml
pH – 6.8.

RESULTS AND DISSUSION

It is not worthy that of the nearly 3,00,000 plant species that exists on earth each individual plant is host to one or more endophytes[13]. Since the number of plant species in the world is so great creative and imaginative stratergies must be used to quickly narrow the search for endophytes displaying various bioactivities. With this view by considering the several reasonable hypothesis. The present investigation was attempted for isolation of endophytic bacteria from mangrove plant, pichavaram, Tamilnadu, India.

In the present study mangrove samples (root, stem and leaf) were collected from pichavaram mangrove forest, selective pretreatment is a prerequisite for the isolation of endophytic microbes. Pretreatment method described by C.Arunachalam and Gayathri.P (2010), was followed in the study[14]. Totally 24 bacterial strains were recovered from all the samples. Up on that 9 from root, 8 from stem and 7 from leaf. All the bacterial strains are markable different from terrestrial bacterial isolates. Pinpointed to larger spread colonies were observed so far these are numerous reports are available on endophytic fungi in mangroves[15,16]. In India also countable number of report showed on diversity of endophytic bacteria, fungi in medicinal plants[17], but from available literature there is only few reports on endophytic bacteria from mangrove particularly in Tamilnadu.

Endophytes are the chemical synthesizers with in plants. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proved for useful drug discovery. Up to now most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents antivirals, antidiabetic agents and other bioactive compounds by their different functional roles[18-20]. In the present study antimicrobial activity of endophytic bacteria were tested by agar well diffusion method. Out of 24 isolates, 6 isolates from root, 2 isolates from stem and 2 isolates from leaf showed broad spectrum activity against clinical pathogens. Root isolates showed good antagonistic activity than stem and root isolates.

Table No.1: Antibacterial activity of Endophytes isolated from mangrove root

S.No	Culture	*S. aureus*	*Strep. pyogens*	*E. coli*	*klebsiella*	*S. typhi*	*Ps. aeurogenosa*	*Ps. fluroscenes*	*Pr. vulgaris*	*Pr. mirabilis*
1	M1R1	+	+	+	+	+	+	+	-	-
2	M1R2	-	-	+	-	-	-	+	-	-
3	M2R1	-	-	+	+	+	+	+	+	+
4	M2R2	+	+	+	+	+	+	+	+	+
5	M3R1	+	-	+	-	+	-	-	-	-
6	M3R2	-	-	-	-	-	+	+	-	-
7	M3R3	+	-	+	+	+	+	+	+	-
8	M3R4	+	+	+	-	+	+	+	+	+
9	M4R1	+	-	+	+	+	-	+	-	+

Table No.2: Antibacterial activity of Endophytes isolated from mangrove stem

S.No	Culture	*S. aureus*	*Strep. pyogens*	*E. coli*	*klebsiella*	*S. typhi*	*Ps. aeurogenosa*	*Ps. fluroscenes*	*Pr. vulgaris*	*Pr. mirabilis*
1	M1S1	-	-	+	-	-	-	-	-	-
2	M1S2	+	-	+	+	-	+	+	-	-
3	M2S1	-	-	+	-	+	-	-	-	-
4	M2S2	-	-	-	-	-	-	+	+	+
5	M3S1	-	+	-	-	-	-	-	-	-
6	M3S2	+	+	+	+	+	+	+	+	+
7	M4S1	-	-	+	+		-	+	+	-
8	M4S2	-	-	-	-	+	-	-	-	-

Table No.3: Antibacterial activity of Endophytes isolated from mangrove leaf

S.No	Culture	*S. aureus*	*Strep. Pyogens*	*E. coli*	*klebsiella*	*S. typhi*	*Ps. aeurogenosa*	*Ps. fluroscenes*	*Pr. vulgaris*	*Pr. mirabilis*
1	M1L1	-	-	-	-	+	-	-	+	+
2	M1L2	+	+	+	+	+	+	+	-	-
3	M2L1	-	-	-	-	-	-	-	-	-
4	M2L2	-	-	-	-	-	-	-	-	-
5	M3L1	+	+	+	-	+	+	+	+	+
6	M4L1	-	-	-	-	-	-	-	-	-
7	M4L2	+	-		+	-	-	+	-	-

CONCLUSION

The present study concluded that this work may be the first report on endophytic bacteria from pichavaram in our state Tamilnadu. This study evidence that mangrove are the potential but under exploited resource for bioactive endophytic bacteria. This study showed promising antimicrobial activity against 9 different clinical pathogens. Detailed investigations on mangrove endophytic bacteria were needed to prove its potential further and it will leads to discovery of numerous high value metabolites. This is suggested for further work.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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